

**What is claimed is**

1. A method for continuously expressing multiple recombinant protein in the milk of transgenic non-human mammals, comprising the steps of:

(a) constructing expression plasmid that carries multiple recombinant protein genes and can express in mammary glands; and

5 (b) transferring said expression plasmid carrying multiple recombinant protein genes by means of gene injection and embryonic implantation to a non-human mammal so that multiple recombinant proteins are expressed in the milk of said transgenic mammal and continue to be expressed during the lactation of the mammal.

10 2. The method according to Claim 1, wherein in said step (a), multiple recombinant protein genes are constructed on the same expression plasmid or respectively on different plasmids.

3. The method according to Claim 1, wherein if multiple recombinant protein genes are constructed on different expression plasmids, a step of mixing different expression plasmids is further added between step (a) and step (b).

15 4. The method according to Claim 1, wherein the construct of said expression plasmid containing multiple recombinant protein genes consists of :

5' regulatory sequence having expression specificity to mammary glands and capable of regulating recombinant protein genes to obtain continuous and stable gene expression in the transgenic mammal during its lactation; and

20 recombinant protein genes that are located behind the regulatory sequence and subject to its control in expression.

5. The method according to Claim 1, wherein said transferred multiple recombinant protein genes are passed onto offsprings through sexual reproduction.

6. The method according to Claim 1, wherein said multiple recombinant protein

genes include human clotting factor IX gene and lactoferrin gene.

7. The method according to Claim 6, wherein the mixture ratio of expression plasmid carrying human clotting factor IX gene and expression plasmid carrying lactoferrin gene is 1:1.

5           8. The method according to Claim 4, wherein said 5' regulatory sequence is bovine  $\alpha$ -lactalbumin promoter.

10           9. The method according to Claim 1, wherein the expression of exogenous porcine lactoferrin in the transgenic milk act as immune modulator, which can help boost the immunity and resistance of nursing offsprings, reducing their diarrhea condition and fighting inflammation.

10. The method according to Claim 1, wherein the expression level of human clotting factor IX in the milk of said transgenic mammal can reach 200-500  $\mu\text{g/mL}$ , and its activity can reach 90% of normal human plasma after purification.

15           11. The method according to Claim 1, wherein said transgenic mammal can express recombinant proteins continuously and stably during lactation.

12. The method according to Claim 1, wherein said transgenic mammal is cow, sheep or swine.

13. The method according to Claim 12, wherein said transgenic mammal is swine.

20           14. The method according to Claim 1, wherein steps are further added after step (b):

(c) collecting said milk containing multiple recombinant proteins; and

(d) isolating multiple recombinant proteins from the aforesaid milk, so as to obtain multiple recombinant proteins from the milk of transgenic mammal.

15. A method for continuously expressing human clotting factor IX and porcine

lactoferrin in the milk of transgenic non-human mammals, comprising the steps of:

(a) constructing expression plasmid that carries human clotting factor IX gene and porcine lactoferrin gene and can express in mammary glands; and

5 (b) transferring said expression plasmid carrying human clotting factor IX gene and porcine lactoferrin gene by means of gene injection and embryonic implantation to a non-human mammal so that said human clotting factor IX and porcine lactoferrin are expressed in the milk of said transgenic mammal and continue to be expressed during the lactation of the mammal.

10 16. The method according to Claim 15, wherein in step (a), human clotting factor IX gene and porcine lactoferrin gene are constructed on the same expression plasmid or respectively on different plasmids;

15 17. The method according to Claim 16, wherein if the human clotting factor IX and porcine lactoferrin are respectively constructed different plasmids, a step of mixing the expression plasmids respectively carrying human clotting factor IX and porcine lactoferrin is further added between step (a) and step (b).

18. The method according to Claim 17, wherein the mixture ratio of said expression plasmid carrying human clotting factor IX and porcine lactoferrin respectively is 1:1.

20 19. The method according to Claim 15, wherein the construct of said expression plasmid containing human clotting factor IX gene and/or porcine lactoferrin gene consists of:

25 5' regulatory sequence having expression specificity to mammary glands and capable of regulating genes behind it to obtain continuous and stable expression of the gene(s) in the transgenic mammal during its lactation; and human clotting factor IX gene and/or porcine lactoferrin gene that are located behind the 5' regulatory sequence and subject to its control in expression.

20. The method according to Claim 19, wherein said 5' regulatory sequence is bovine  $\alpha$ -lactalbumin promoter.

21. The method according to Claim 15, wherein said transferred human clotting factor IX gene and porcine lactoferrin gene are passed onto offsprings through sexual reproduction.

22. The method according to Claim 15, wherein the expression level of human clotting factor IX in the milk of said transgenic mammal can reach 200-500  $\mu\text{g/mL}$ , and its activity can reach 90% of normal human plasma after purification.

23. The method according to Claim 15, wherein said transgenic mammal can express recombinant proteins continuously and stably during lactation.

24. The method according to Claim 15, wherein said transgenic mammal is cow, sheep or swine.

25. The method according to Claim 24, wherein said transgenic mammal is swine.

26. The method according to Claim 15, wherein the expression of exogenous porcine lactoferrin in the transgenic milk act as immune modulator, which can help boost the immunity and resistance of nursing offsprings, reducing their diarrhea condition and fighting inflammation.

27. The method according to Claim 15, wherein steps are further added after step (b):

(c) collecting the milk containing human clotting factor IX and porcine lactoferrin; and

(d) isolating the human clotting factor IX or porcine lactoferrin from said milk, so as to obtain human clotting factor IX or porcine lactoferrin from the milk of transgenic mammal.